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(71) Applicant *(for all designated States except US)*: A+ SCIENCE INVEST AB [SE/SE]; P.O. Box 3096, S-400 10 Göteborg (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: OLMARKER, Kjell [SE/SE]; Gustavsgatan 35, S-431 66 Mölndal (SE). RYDEVIK, Björn [SE/SE]; Laboratorietrappan 6, S-412 68 Göteborg (SE).

(74) Agent: GÖTEBORGS PATENTBYRÅ DAHLS AB; Sjöporten 4, S-417 64 Göteborg (SE).

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(54) Title: USE OF CERTAIN DRUGS FOR TREATING NERVE ROOT INJURY

(57) Abstract

The present invention relates to pharmaceutical compositions for the treatment of spinal disorders caused by the liberation of TNF-α comprising an effective amount of a TNF-α inhibitor, as well as method for treatment of such disorders, and the use of TNF-α inhibitors in the preparation of pharmaceutical composition for such treatment.

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TITLE

USE OF CERTAIN DRUGS FOR TREATING NERVE ROOT INJURY

DESCRIPTION

Technical field

The present invention relates to the use of a TNF-α inhibitor in the preparation of pharmaceutical compositions for the treatment of nerve root injury, as well as a method for treating nerve root injury.

The object of the present invention is to obtain a possibility to treat nerve root injury induced by disk herniation, which may turn up as radiating pain into the arm or leg (sciatica), by blocking disk related cytokines.

Background of the invention

Disk herniation is a troublesome disorder, which can cause pronounced pain and muscle dysfuntion, and thereby loss of ability to work. A herniation may occur in any disk in the spine but herniations in the lumbar and the cervical spine are most common. A disk herniation in the cervical spine may induce radiating pain and muscle dysfunction in the arm and herniation in the lumbar spine may induce radiating pain and muscle dysfunction in the leg. The radiating pain in the leg is generally referred to as "sciatica". Disk herniation will cause trouble to a varying degree, and the pain may last for one or two months or in severe cases up to 6 months. The arm or leg pain that can occur as a result of disk herniation can be very intense and may thus affect the individual patiet's whole life situation during the sickness period.

US-A-5,703,092 discloses the use of hydroxamic acid compounds and carbocyclic acids as metalloproteinase and TNF inhibitors, and in particular in treatment of arthritis and other related inflammatory diseases. No use of these compounds for the treatment of nerve root injuries is disclosed or hinted at.

US-A-4,925,833 discloses the use of tetracyclines to enhance bone protein synthesis, and treatment of osteoporosis.

US-A-4,666,897 discloses inhibition of mammalian collagenolytic enzymes by tetracyclines. The collagenolytic activity is manifested by excessive bone resorption, periodontal disease, rheumatoid arthritis, ulceration of cornea, or resorption of skin or other connective tissue collagen.

Neither of these latter two documents mentions nerve root injury or the treatment thereof.

Description of the present invention

It has now surprisingly been shown possible to be able to treat nerve root injuries, or at least alleviate the symptoms of nerve root injuries by using a pharmaceutical composition comprising an therapeutically active amount of a TNF-α inhibitor selected from the group consisting of metalloproteinase inhibitors excluding methylprenisolone, tetracyclines including chemically modified tetracyclines, quinolones, corticosteroids, thalidomide, lazaroides, pentoxyphylline, hydroxamic acid derivatives, napthopyrans, soluble cytokine receptors, monoclonal antibodies towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, lactoferrin and lactoferrin derived analogous, and melatonin in the form of bases or addition salts together with a pharmaceutically acceptable carrier.

The therapeutically effective amount is a dosage normally used when using such compounds for other therapeutic uses. Many of these drugs are commercially known registered drugs.

Compounds that possess this activity are tetracyclines, such as tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, and chemically modified tetracyclines dedimethylaminotetracycline, hydroxamic acid compounds, carbocyclic acids and derivatives, thalidomide, lazaroides, pentoxyphylline, napthopyrans, soluble cytokine receptors, monoclonal antibodies towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, lactoferrin and lactoferrin derived analogous, melatonin, norfloxacine, ofloxacine, ciprofloxacine, gatifloxacine, pefloxacine, lomefloxacine, and temafloxacine. These can be present as bases or in the form of addition salts, whichever possesses the best pharmaceutical effect, and best property to be brought into a pharmaceutical suitable composition.

Further, the active component comprises a substance inhibiting a compound trigged by the

release of TNF-α, such as interferon-gamma, interleukin-1, and nitrogen oxide (NO) in the form of base or addition salts.

The invention further relates to a method for inhibiting the symptoms of nerve root injury.

The effects of doxycycline, soluble cytokine-receptors, and monoclonal cytokine-antibodies have been studied and the methods used and results obtained are disclosed below.

Example

Study design.

The effects of nucleus pulposus and various treatments to block TNF-α activity were evaluated in an experimental set-up using immunohistochemistry and nerve conduction velocity recordings.

Summary of background data:

A meta-analysis of observed effects induced by nucleus pulposus reveals that these effects might relate to one specific cytokine, Tumor Necrosis Factor alpha (TNF(α).

Objectives.

To assess the presence of TNF(α) in pig nucleus pulposus cells and to see if blockage of TNF(α) also blocks the nucleus pulposus-induced reduction of nerve root conduction velocity.

Methods

Series-1: Cultured nucleus pulposus-cells were immunohistologically stained with a monoclonal antibody for TNF(α).

Series-2: Nucleus pulposus was harvested from lumbar discs and applied to the sacro-coccygeal cauda equina in 13 pigs autologously. Four pigs received 100 mg of doxycycline intravenously, 5 pigs had a blocking monoclonal antibody to TNF-α applied locally in the nucleus pulposus, and 4 pigs remained non-treated and formed control. Three days after the application the nerve root conduction velocity was determined over the application zone by local electrical stimulation.

Series-3: Thirteen pigs had autologous nucleus pulposus placed onto their sacrococcygeal

cauda equina similar to series-2. Five pigs (bodyweight 25 kg) received Remicade[R] (infliximab) 100 mg i.v. preoperatively, and 8 pigs received Enbrel[R] (etanercept) 12.5 mg s.c. preoperatively and additionally 12.5 mg s.c. three days after the operation. Seven days after the nucleus pulposus-application the nerve root conduction velocity was determined over the application zone by local electrical stimulation according to series-2.

Results.

Series-1: TNF-α was found to be present in the nucleus pulposus-cells.

Series-2: The selective antibody to TNF-α limited the reduction of nerve conduction velocity, although not statistically significantly to the control series. However, treatment with doxycycline significantly blocked the nucleus pulposus-induced reduction of conduction velocity.

Series-3: Both drugs (infliximab, and etanercept) blocked the nucleus pulposus induced nerve injury efficiently and normal average nerve conduction velocities were found after treatment with both of these two drugs.

Conclusion.

For the first time a specific substance, Tumor Necrosis Factor-alpha, has been linked to the nucleus pulposus-induced effects of nerve roots after local application. Although the effects of this substance may be synergistic with other similar substances, the data of the present study may be of significant importance for the continued understanding of nucleus pulposus' biologic activity, and might also be of potential use for future treatment strategies of sciatica.

After previously being considered as just a biologically inactive tissue component compressing the spinal nerve root at disc herniation, the nucleus pulposus has recently been found to be highly active, inducing both structural and functional changes in adjacent nerve roots when applied epidurally (24,37,38,41,42). It has thereby been established that autologous nucleus pulposus may induce axonal changes and a characteristic myelin injury (24,38,41,42), increased vascular permeability (9,44), intra vascular coagulation (24,36), and that membrane-bound structure or substances of the nucleus pulposus-cells are responsible for these effects (24,37). The effects have also been found to be efficiently blocked by methyl-prednisolone and cyclosporin A (2,38). When critically looking at these data, one realizes that there is at least one cytokine that relates to all of these effects, Tumor Necrosis

Factor alpha (TNF-α). To assess if TNF-α may be involved in the nucleus pulposus induced nerve root injury the presence of TNF-α in nucleus pulposus-cells was assessed and was studied if the nucleus pulposus-induced effects could be blocked by doxycycline, a soluble TNF-receptor, and a selective monoclonal TNF-antibody, the latter administered both locally in the nucleus pulposus and systemically.

MATERIAL AND METHODS

Series-1, Presence of TNF-α in pig nucleus pulposus-cells:

Nucleus pulposus (NP) from a total of 13 lumbar and thoracic discs were obtained from a pig used for other purposes. NP was washed once in Ham's F12 medium (Gibco BRL, Paisley, Scotland) and then centrifuged and suspended in 5 ml of collagenase solution in Ham's F12 medium (0.8 mg/ml, Sigma Chemical Co., St Louis, MO, USA) for 40 minutes, at 37°C in 25 cm^2 tissue culture flasks. The separated NP-cell pellets were suspended in DMEM/F12 1:1 medium (Gibco BRL, Paisley, Scotland) supplemented with 1% L-glutamine 200 mM (Gibco BRL, Paisley, Scotland), 50μg/ml gentamycine sulphate (Gibco BRL, Paisley, Scotland) and 10% foetal calf serum (FCS), (Gibco BRL, Paisley, Scotland). The cells were cultured at 37°C and 5% $C0_2$ in air for 3-4 weeks and then cultured directly on tissue culture treated glass slides (Becton Dickinson & Co Labware, Franklin Lakes, NJ, USA). After 5 days on the glass slides, the cells were fixed in situ by acetone for 10 minutes. After blocking irrelevant antigens by application of 3% H_2O_2 (Sigma Chemical Co., St Louis, MO, USA) for 30 minutes and Horse Serum (ImmunoPure ABC, peroxidase mouse IgG staining kit nr.32028, Pierce, Rockford, IL) for 20 minutes, the primary antibody (Anti-pig TNF-α monoclonal purified antibody, Endogen, Cambridge, MA, USA) was applied over night at +40°C, diluted at 1:10, 1:20 and 1:40. For control, BSA (bovine serum albumin, Intergen Co, New York, USA) suspended in PBS (phosphate buffered saline, Merck, Darmstadt, Germany) was applied in the same fashion. The next day the cells were washed with 1% BSA in PBS and the secondary antibody (ImmunoPure ABC, peroxidase mouse IgG staining kit nr.32028, Pierce, Rockford, IL) was applied for 30 minutes. To enhance this reaction, the cells were exposed to Avidin-Biotin complex for additionally 30 minutes (ImmunoPure ABC, peroxidase mouse IgG staining kit nr.32028, Pierce, Rockford, IL). The cells were then exposed to 20 mg of DAB (3,3-diaminobenzidine tetrahydrochloride nr. D-5905, Sigma Chemical Co., St Louis, MO, USA) and 0.033 ml of 3% H_20_2 in 10 ml of saline for 10 minutes. The cells were washed in PBS, dehydrated in a

series of ethanol, mounted and examined by light microscopy by an unbiased observer regarding the presence of a brown colouration indicating presence of TNF-α.

Series-2, Neurophysiologic evaluation:

Thirteen pigs, (body weight 25-30 kg) received an intramuscular injection of 20 mg/kg body weight of Ketalar[R] (ketamine 50 mg/ml, Parke-Davis, Morris Plains, New Jersey) and an intravenous injection of 4 mg/kg body weight of Hypnodil[R] (methomidate chloride 50 mg/ml, AB Leo, Helsingborg, Sweden) and 0. I mg/kg body weight of Stresnil[R] (azaperon 2 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). Anaesthesia was maintained by additional intravenous injections of 2 mg/kg body weight of Hypnodil[R] and 0.05 mg/kg body weight of Stresnil[R]. The pigs also received an intravenous injection of 0.1 mg/kg of Stesolid Novum[R] (Diazepam, Dumex, Helsingborg, Sweden) after surgery.

Nucleus pulposus was harvested from the 5th lumbar disc through a retro peritoneal approach (42). Approximately 40 mg of the nucleus pulposus was applied to the sacrococcygeal cauda equina through a midline incision and laminectomy of the first coccygeal vertebra. Four pigs did not receive any treatment (no treatment). Four other pigs received an intravenous infusion of 100 mg of doxycycline (Vibramycino, Pfizer Inc., New York, USA) in 100 ml of saline over 1 hour. In 5 pigs, the nucleus pulposus was mixed with 100 gl of a 1,11 mg/ml suspension of the anti-TNF-α antibody used in series 1, before application.

Three days after the application, the pigs were reanaesthetized by an intramuscular injection of 20 mg/kg body weight of Ketalar[R] and an intravenous injection of 35 mg/kg body weight of Pentothal[R] (Thiopental sodium, Abbott lab, Chicago, IL). The pigs were ventilated on a respirator. Anaesthesia was maintained by an intravenous bolus injection of 100 mg/kg body weight of Chloralose (α)-D(+)-gluco-chloralose, Merck, Darmstadt, Germany) and by a continuous supply of 30 mg/kg/hour of Chloralose. A laminectomy from the 4th sacral to the 3rd coccygeal vertebra was performed. The nerve roots were covered with Spongostane[R] (Ferrosan, Demnark). Local tissue temperature was continuously monitored and maintained at 37.5-38.0°C by means of a heating lamp.

The cauda equina was stimulated by two E2 subdermal platinum needle electrodes (Grass

Instrument Co., Quincy, MA) which were connected to a Grass SD9 stimulator (Grass Instrument Co., Quincy, MA) and gently placed intermittently on the cauda equina first 10 mm cranial and then 10 mm caudal to the exposed area. To ensure that only impulses from exposed nerve fibres were registered, the nerve root that exited from the spinal canal between the two stimulation sites were cut. An EMG was registered by two subdermal platinum needle electrodes which were placed into the paraspinal muscles in the tail approximately 10 mm apart. This procedure is reproducible and represents a functional measurement of the motor nerve fibres of the cauda equina nerve roots. The EMG was visualized using a Macintosh IIci computer provided with Superscope software and MacAdios II AID converter (GW Instruments, Sommerville, MA) together with a Grass P18 preamplifier (Grass Instrument Co., Quincy, MA). The separation distance between the first peaks of the EMG from the two recordings was determined and the separation distance between the two stimulation sites on the cauda equina was measured with calipers. The nerve conduction velocity between the two stimulation sites could thus be calculated from these two measurements.

The person performing the neurophysiologic analyses was unaware of the experimental protocol for the individual animal, and after finishing the complete study the data were arranged in the three experimental groups and statistical differences between the groups were assessed by Student's t-test. The experimental protocol for this experiment was approved by the local animal research ethics committee.

Series-3: Thirteen pigs had autologous nucleus pulposus placed onto their sacrococcygeal cauda equina similar to series-2. Five pigs (bodyweight 25 kg) received the human/murine monoclonal antibody Remicade^{R} (infliximab, Immunex Corporation, Seattle, WA 98101, USA) 100 mg i.v. preoperatively, and 8 pigs received Enbrel^{R} (etanercept, Centocor B.V., Leiden, the Netherlands) 12.5 mg s.c. preoperatively and additionally 12.5 mg s.c. three days after the operation. Seven days after the nucleus pulposus-application the nerve root conduction velocity was determined over the application zone by local electrical stimulation according to series-2. To blind the study the neurophysiological evaluation was conducted in parallel to another study and the person performing the analyses did not know from which study and what treatment each specific animal was subjected to. No non-treated animals were included in the series-3 due to the pre-existing knowledge of nerve conduction velocity

after seven days of either nucleus pulposus or fat (control) application. The statistical difference between the groups, infliximab, and etanercept, nucleus pulposus without treatment (positive control from previous data) and application of retroperitoneal fat (negative control from previous data) was assessed by using ANOVA and Fisher's PLSD at 5%.

RESULTS

Series-1, Presence of TNF-α in pig nucleus pulposus-cells:

Examples of the light microscopic appearance of the stained glass slides. In the sections using BSA in PBS as "primary antibody" (control) no staining was observed, ensuring that there was no labelling and visualization of irrelevant antigens. When the anti-TNF-α antibody was applied at 1:40 dilution there was only a weak staining. However, the staining increased with diminishing dilutions of the antibody. The staining was seen in the soma of the cells and it was not possible to differentiate whether TNF-α was located in the cytoplasm, on the cell surface bound to the cell-membrane, or both.

Series-2, Neurophysiologic evaluation:

Application of non-modified nucleus pulposus and without any treatment induced a reduction in nerve conduction velocity similar to previous studies (Table 1), whereas treatment with doxycycline completely blocked this reduction ($p<0.01$ Student's t-test). Local application of anti-TNF-α-antibody also induced a partial block of this reduction, although not as complete as doxycycline and not statistically significant to the no treatment-series.

Series-3: Treatment with both drugs seemed to prevent the nucleus pulposus-induced reduction of nerve root conduction velocities since the average nerve conduction velocity for both these treatment groups were close to the average conduction of fat-application series as seen in a previous study (Table 2). There was a statistically significant difference to application of nucleus pulposus, but without any treatment, seen for both drugs.

Table 1 - Series-2

Treatment	n	NCV(m/s±SD)
Local anti-TNF-α	5	64±28
Doxycycline	4	76± 9
No treatment	4	46±12

Table 2 - Series-3

Treatment	n	NCV(m/s+SD)
*Fat**	*5*	*76±11*
Embrel[R]	8	78±14
Remicade[R]	5	79±15
*No treatment**	*5*	*45±19*

* Data included from ref. no. 42, Olmarker et al, 1993

DISCUSSION

The data of the present study demonstrated that TNF-α may be found in nucleus pulposus-cells of the pig. If TNF-α was blocked by a locally applied selective monoclonal antibody, the nucleus pulposus-induced reduction of nerve root conduction velocity was partially blocked, although no statistically significant compared to the series with non-treated animals. However, if systemic treatments with doxycycline, infliximab, and etanercept were used to inhibit TNF-α, the reduction of nerve conduction velocity was significantly prevented.

In recent years, it has been verified that local application of autologous nucleus pulposus may injure the adjacent nerve roots. Thus, it has become evident that the nerve root injury seen at disc herniation may not be solely based on mechanical deformation of the nerve root, but may also be induced by unknown "biochemical effects" related to the epidural presence of herniated nucleus pulposus. Although this new research field has generated many experimental studies, the mechanisms and substances involved are not fully known. It has been seen that local application of autologous nucleus pulposus may induce axonal injury (24,37,38,40-42), a characteristic injury of the myelin sheath (24,38,40-42), a local increase of vascular permeability (9,36,44), intra vascular coagulations, reduction of intra neural blood flow (43), and leukotaxis (36). It has been seen that the nucleus pulposus-related effects may be blocked efficiently by methylprednisolone (38) and cyclosporin A (2), and slightly less efficiently by indomethacin (3), and lidocaine (69). Further, it has been understood that the effects are mediated by the nucleus pulposus-cells (37), particularly by

substances or structures bound to the cell-membranes (25). When critically considering these data, it becomes evident that at least one specific cytokine could be related to these observed effects, Tumor Necrosis Factor-alpha (TNF-α). TNF-α may induce nerve injury (29,31,45,50,66) mainly seen as a characteristic myelin injury that closely resembles the nucleus pulposus-induced myelin-injury (29,47,51,54,62,64,66,70). TNF-α may also induce an increase in vascular permeability (47,66) and initiate coagulation (22,34,63). Further, TNF-α may be blocked by steroids (4,8,21,61,68), and cyclosporin A (11,55,67,68). However, the blocking effect on TNF-α is not so pronounced by NSAID (14,17,20) and very low or the opposite by lidocaine (5,32,46,60). It was recently observed that local application of nucleus pulposus may induce pain-related behaviour in rats, particularly thermal hyperalgesia (23,40). TNF-α has also been found to be related to such pain-behaviouristic changes (12,35,56,66), and also to neuropathies in general (30,54,56,57). However there are no studies that have assessed the possible presence of TNF-α in the cells of the nucleus pulposus.

To assess if TNF-α could be related to the observed nucleus pulposus induced reduction in nerve root conduction velocity it was necessary first to analyse if there was TNF-α in the nucleus pulposus-cells. The data clearly demonstrated that TNF-α was present in these cells. TNF-α is produced as precursor (pro-TNF) that is bound to the membrane and it is activated by cleavage from the cell-membrane by a zinc-dependent metallo-endopeptidase (TNF-α converting enzyme, TACE) (6,15,16,48,49). This may thus relate well to experimental findings where application of the mere cell-membranes of autologous nucleus pulposus-cells induced nerve conduction velocity reduction, which indicated that the effects were mediated by a membrane-bound substances. Second, the effects of the TNF-α had to be blocked in a controlled manner. We then first choose to add the same selective antibody that was used for immunohistochemistry in series 1, which is known to also block the effects of TNF-α, to the nucleus pulposus before application. Also, we choose to treat the pigs with doxycycline, which is known to block TNF-α (26,27,33,52,53). However, due to the low pH of the doxycycline preparation it was chosen to treat the pigs by intravenous injection instead of local addition to the nucleus pulposus since nucleus pulposus at a low pH has been found to potentiate the effects of the nucleus pulposus (38,39).

Two recently developed drugs for specific TNF-α inhibition were also included in the study.

Infliximab is a chimeric monoclonal antibody composed of human constant and murine variable regions, and binds specifically to human TNF-α. As opposed to the monoclonal antibody used in series-2 for the 3 days observation period, infliximab was not administered locally in the autotransplanted nucleus pulposus but instead systemically in a clinically recommended dose (4 mg/kg). Etanercept is a dimeric fusion protein consisting of the Fc portion of human IgG. The drug was administered in a dosage comparable to the recommended dose for pediatric use (0.5 mg/kg, twice a week).

The data regarding nerve conduction velocity showed that the reduction was completely blocked by the systemic-treatment and that the nerve conduction velocities in these series were close to the conduction velocity after application of a control substance (retro peritoneal fat) from a previous study (42). Application of the anti-TNF-α-antibody to the nucleus pulposus also partially prevented the reduction in nerve conduction velocity, however, not as pronounced as doxycycline, and the velocity in this series was not statistically different to the velocity in the series with not treated animals, due to the wide deviation of the data.

The fact that the local anti-TNF-α antibody treatment only partially blocked the nucleus pulposus-induced reduction of nerve conduction velocity and the high standard deviation of the data could probably have at least three different explanations. First, if looking at the specific data within this group it was found that the nerve conduction velocity was low in 2 animals (mean 37.5 m/s) and high in 3 animals (mean 81.3 m/s). There are thus 2 groups of distinctly different data within the anti-TNF-α treatment series. This will account for the high standard deviation and might imply that the blocking effect was sufficient in 3 animals and non-sufficient in 2 animals. The lack of effects in these animals could be based simply on the amount of antibodies in relation to TNF-α molecules not being sufficient, and if a higher dose of the antibody had been used, the TNF-α effects would thus have been blocked even in these animals. Such a scenario could then theoretically imply that TNF-α alone is responsible for the observed nucleus pulposus-induced effects, and that this could not be verified experimentally due to the amount of antibody being too low.

Second, it is also known that tetracyclines such as doxycycline and minocycline may block a number of cytokines and other substances. For instance they may block IL-1 (1,28,58),

IFNγ (27), NO-synthetasel, and metalloproteinases (l,53,58). Particularly IL-1 and IFNγ are known to act synergistically with TNF-α and are known to be more or less neurotoxic (7,10,13,18,19,56,59). These substances are also blocked by steroids and cyclosporin A which corresponds well with the previous observations on nucleus pulposus-induced nerve root injury which have shown that the nucleus pulposus-induced effects may be blocked by these substances (8,67). One may therefore also consider the possibility that a selective block of TNF-α may not be sufficient to completely block the nucleus pulposus-induced effects on nerve function, and that simultaneous block of other synergistic substances is necessary as well. Thus, this scenario, on the other hand, implies that TNF-α is not solely responsible for the nucleus pulposus-induced effects, and that other synergistic substances, which are also blocked by doxycycline, may be necessary.

The third explanation could be that the amount of TNF in the nucleus pulposus may well be enough to start the pathophysiologic cascade locally in the nerve root, comprising increased vascular permeability and aggregation and recruitment of systemic leukocytes. However, it is these leukocytes that have the major content of TNF-α and that systemic treatment in a sufficient dose is necessary to block the contribution from these leukocytes, and thereby also blocking the events leading to nerve injury.

TNF-α may have various pathophysiologic effects. It may have direct effects on tissues such as nerve tissue and blood vessels, it may trigger other cells to produce other pathogenic substances and it may trigger release of more TNF-α both by inflammatory cells and also by Schwann-cells locally in the nerve tissue (65).]here is thus reason to believe that even low amounts of TNF-α may be sufficient to initiate these processes and that there is a local recruitment of cytokine producing cells and a subsequent increase in production and release of other cytokines as well as TNF-α. TNF-α may therefore act as the "ignition key" of the pathophysiologic processes and play an important role for the initiation of the pathophysiologic cascade behind the nucleus pulposus-induced nerve injury. However, the major contribution of TNF-α may be derived from recruited, aggregated and maybe even extravasated leukocytes, and that successful pharmacologic block may be achieved only by systemic treatment.

In conclusion, although the exact role of TNF-α can not be fully understood from the

experimental set-up, we may conclude that for the first time a specific substance (TNF-α) has been linked to the nucleus pulposus-induced nerve root injury. This new information may be of significant importance for the continued understanding of nucleus pulposus-induced nerve injury as well as raising the question of the potential future clinical use of pharmacological interference with TNF-α and related substances, for treatment of sciatica.

The presence of TNF-α in pig nucleus pulposus-cells was thus immunohistochemically verified. Block of TNF-α by a locally applied monoclonal antibody partially limited the nucleus pulposus-induced reduction of nerve root conduction velocity, whereas intravenous treatment with doxycycline, infliximab, and etanercept significantly blocked this reduction. These data for the first time links one specific substance, TNF-α, to the nucleus pulposus-induced nerve injury.

Aminoguanidine has showed to inhibit the release of nitrogen oxide (NO) at nerve root injuries by inhibiting inducible nitrogen oxide synthetase, and aminoguanidine is thus one compound that inhibits a compound trigged by the release of TNF-α.

The compounds of the invention can be administered in a variety of dosage forms, e.g, orally, in the form of tablets, capsules, sugar or film coated tablets, liquid solutions; rectally, in the form of suppositories; parenterally, e.g., intramuscularly or by intravenous injection or infusion. The therapeutic regimen for the different clinical syndromes must be adapted to the type of pathology taken in to account, as usual, also the route of administration, the form in which the compound is administered and age, weight, and condition of the subject involved.

The oral route is employed, in general, for all conditions, requiring such compounds. In emergency cases preference is given to intravenous injection. For these purposes the compounds of the invention can be administered orally at doses ranging from about 20 to about 1500 mg/day. Of course, these dosage regimens may be adjusted to provide the optimal therapeutic response.

The nature of the pharmaceutical composition containing the compounds of the invention in association with pharmaceutically acceptable carriers or diluents will, of course, depend upon the desired route of administration. The composition may be formulated in the

conventional manner with the usual ingredients. For example, the compounds of the invention may be administered in the form of aqueous or oily solutions or suspensions, tablets, pills, gelatine capsules (hard or soft ones)syrups, drops or suppositories.

Thus for oral administration, the pharmaceutical compositions containing the compounds of the invention are preferably tablets, pills or gelatine capsules, which contain the active substance together with diluents, such as lactose, dextrose, sucrose, mannitol, sorbitol, cellulose; lubricants, e.g., silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; or they may also contain binders, such as starches, gelatine, methyl cellulose, carboxymethylcellulose, gum arabic, tragacanth, polyvinylpyrrolidone; disaggregating agents such as starches, alginic acid, alginates, sodium starch glycolate, microcrystalline cellulose; effervescing agents such a carbonates and acids; dyestoffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and in general non-toxic and pharmaceutically inert substances used in the formulation of pharmaceutical compositions. Said pharmaceutical compositions may be manufactured in known manners, e.g., by means of mixing, granulating, tableting, sugar-coating or film-coating processes. In the case film providing compounds can be selected to provide release in the right place in the intestinal tract with regard to absorption and maximum effect. Thus pH-dependent film formers can be used to allow absorption in the intestines as such, whereby different phthalate are normally used or acrylic acid/methacrylic acid derivatives and polymers.

The liquid dispersions for oral administration may be e.g., syrups, emulsion, and suspensions.

The syrups may contain as carrier, e.g., saccharose, or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, e.g., a natural gum, such as gum arabic, xanthan gum, agar, sodium alginate, pectin, methyl cellulose, carboxymethylcellulose, polyvinyl alcohol.

The suspension or solutions for intramuscular injections may contain together with the active compound, a pharmaceutically acceptable carrier, such as e.g., sterile water, olive oil,

ethyl oleate, glycols,, e.g., propylene glycol, and if so desired, a suitable amount of lidocaine hydrochloride. Adjuvants for trigging the injection effect can be added as well.

The solutions for intravenous injection or infusion may contain as carrier, e.g., sterile water, or preferably, a sterile isotonic saline solution, as well as adjuvants used in the field of injection of active compounds.

The suppositories may contain together with the active compound, a pharmaceutically acceptable carrier, e.g., cocoa-butter polyethylene glycol, a polyethylene sorbitan fatty acid ester surfactant or lecithin.

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CLAIMS

1. Use of a TNF-α inhibitor selected from the group consisting of metallo proteinase inhibitors excluding methylprenisolone, tetracyclines including chemically modified tetracyclines, quinolones, corticosteroids, thalidomide, lazaroides, pentoxyphyllines, hydroxamic acid derivatives, carbocyclic acids, napthopyrans, soluble cytokine receptors, monoclonal antibodies towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, lactoferrin and lactoferrin derived analogous, and melatonin in the form of the base or its addition salt, in the preparation of a pharmaceutical composition for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α and compounds trigged by the liberation of or presence of TNF-α by inhibiting spinal disk TNF-α .

2. Use according to claim 1, wherein the active component is selected from the group consisting of tetracycline, doxycycline, lymecycline, oxytetracycline, minocycline, and chemically modified tetracyclines dedimethylaminotetracycline, in the form of bases or addition salts.

3. Use according to claim 2, wherein the active component is doxycycline.

4. Use according to claim 1, wherein the active component is selected from hydroxamic acid compounds, carbocyclic acids and derivatives, thalidomide, lazaroides, pentoxyphylline, napthopyrans, soluble cytokine receptors, monoclonal antibody towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, melatonin in the form of bases or addition salts.

5.Use according to claim 1, wherein the active component is selected from norfloxacine, ofloxacine, ciprofloxacine, gatifloxacine, pefloxacine, lomefloxacine, and temafloxacine in the form of bases or addition salts.

6.Use according to claim 1, wherein the active component is a metallo proteinase inhibitor in the form of base or addition salts.

7.Use according to claim 1, wherein the active component comprises a substance inhibiting a

compound trigged by the release of TNF-α, such as interferon-gamma, interleukin-1, and nitrogen oxide (NO) in the form of base or addition salts

8. Method for the treatment of spinal disorders as nerve root injury caused by the liberation of TNF-α in mammals, including man, comprising the administration of a pharmaceutically effective amount of a TNF-α inhibitor selected from the group consisting of metallo proteinase inhibitors excluding methylprednisolone, tetracyclines including chemically modified tetracyclines, quinolones, corticosteroids, thalidomide, lazaroides, pentoxyphylline, hydroxamic acid derivatives, carbocyclic acids, napthopyrans, soluble cytokine receptors, monoclonal antibodies towards TNF-α, amrinone, pimobendan, vesnarinone, phosphodiesterase III inhibitors, lactoferrin and lactoferrin derived analogous, and melatonin in the form of the base or its addition salt.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01671

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/65, A61K 31/56, A61K 31/33, A61K 31/16, A61K 31/19, A61P 25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File MEDLINE, MEDLINE accession no. 97218877, Document no. 97218877, Schlumpf U et al: "Acute lumbar disk displacement with nerve root compression. Indications for peridural steroid injection."; SCHWEIZERISCHE RUNDSCHAU FUR MEDIZIN PRAXIS, (1997 Feb 18) 86 (8) 292-5 --	1,6-8
X	STN International, File MEDLINE, MEDLINE accession no. 76231109, Document no. 76231109, Schenk S et al: "Intrathecal cortison injection in lumbar disc problems"; ARCHIV FUR ORTHOPADISCHE UND UNFALL-CHIRURGIE, (1976 Jun 18) 85 (1) 21-31 --	1,6-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 2000	29 -01- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Solveig Gustavsson/ELY Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01671

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File EMBASE, EMBASE accession no. 97339851, Document no. 1997339851, Kraemer J. et al: "Lumbar epidural perineural injection: A new technique"; European Spine Journal, (1997) 6/5 (357-361) --	1,6-8
X	STN International, File MEDLINE, MEDLINE accession no. 95064276, Document no. 95064276, Olmarker K. et al: "Effects of methylprednisolone on necleus pulposus-induced nerve root injury"; SPINE (1994 Aug 15) 19 (16) 1803-8 --	1,6-8
X	STN International, File MEDLINE, MEDLINE accession no. 96310878, Document no. 96310878, Pennica, D. et al: "Cardiotrophin-1, a cytokine present in embryonic muscle, supports long-term survival of spinal motoneurons"; NEURON, (1996 Jul) 17 (1) 63-74 --	1,7-8
X	STN International, File MEDLINE, MEDLINE accession no. 1998068711, Document no. 98068711, Sommer C. et al: "A metallprotease-inhibitor reduces pain associated behavior in mice with experimental neuropathy"; NEUROSCIENCE LETTERS, (1997 Nov 14) 237 (1) 45-8 --	1,4,6-8
X	STN International, File MEDLINE, MEDLINE accession no. 1998173500, Document no. 98173500, Sommer C. et al: "The effect of thalidomide treatment on vascular pathology and hyperalgesia caused by chronic constriction injury of rat nerve"; PAIN, (1998 Jan) 74 (1) 83-91 --	1,4,6-8
P,X	STN International, File MEDLINE, MEDLINE accession no. 1999071916, Document no. 99071916, Olmarker K. et al: "Tumor necrosis factor alpha and nucleus-pulposus-induced nerve root injury"; SPINE, (1998 Dec 1) 23 (23) 2538-44 -- --------	1-3,6-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/01671

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8
   because they relate to subject matter not required to be searched by this Authority, namely:

   See extra sheet.

2. ☒ Claims Nos.: 1 and 7-8
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   It is not clear from the wording of the claims, what is excluded from or included in the claimed invention.

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claim 8 relate to a method of treatment of the human or animal body by surgery or by therapy/a diagnostic method practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compound(s)/composition(s).